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(57) Abstract

Disclosed herein are compounds, compositions, and methods to inactivate a virus and destroy tumor cells. The methods involve the addition into the cell of a compound containing a photosensitizing chemical and an energy donating chemical, optionally linked by a chemical tether. Also introduced into the cell are means to chemically active the energy donating chemical which photoactivates es the photosensitizing chemical which then destroys the tumor or virus. The photosensitizing chemical is preferably hypericin, porphyrin, or an analog and the energy donating chemical is preferably luciferin or an analog. Methods for synthesizing the chemicals are also disclosed. Further, the energy donating chemical is activated by an activating chemical. Expression of the activating chemical is regulated so that it is only expressed in the virus-infected or tumor cells. Regulating the activating chemical is accomplished by a number of methods including construction of an expression plasmid containing a gene encoding the activating chemical under control of a promoter which is transactivated by replication of the virus or transactivated by elevated levels of proteins expressed in tumor cells.

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MOLECULAR FLASHLIGHT

Background of the Invention

In recent years, photodynamic therapy (PDT) has emerged as a promising tool in both antiviral and cancer chemotherapy. Photodynamic therapy is possible because some photoactive molecules have an affinity for tumor-infected cells. In the presence of light of the appropriate wavelengths, the photoactive molecules absorb the light and inactivate the virus and destroy the virus-infected cell or tumor cell. Photoactive molecules which are currently employed include a mixture of compounds called hematoporphyrin derivatives (HpD), the purpurins and the phthalocyanines. A current focus is on the preparation of photoactive molecules whose maximum wavelength is in the red region of the spectrum, because tissue penetration is optimum in this region. Indeed, the most common strategy for the generation of new photoactive molecules has simply been the modification of existing ones. A major drawback is that PDT cannot be extended to the treatment of tumors in regions of the body where light does not penetrate.

Moan, "Yearly Review: Porphyrin Photosensitization and Phototherapy," Photochemistry and Photobiology: Vol.

43, No. 6, pp. 681-690 (1986), which is incorporated herein in its entirety by reference, discloses the tumor localizing property of porphyrins and their use in photodynamic cancer therapy. The method of treatment involves direct injection of the photosensitizer followed by possible administration of singlet oxygen scavengers to diminish any possible side effects. The photosensitizer molecules are affected by a light source located outside the body. Moan et al., specifically mention that because the penetration of the red light is limited, photodynamic therapy can never be used to eliminate large tumors.

Meruelo et al., "Therapeutic Agents With Dramatic Antiretroviral Activity and Little Toxicity at Effective Doses: Aromatic Polycyclic Diones Hypericin and Pseudohypericin," Proc. Natl. Acad. Sci., Vol. 85, pp. 5230-5234, July 1988, which is incorporated herein in its entirety by reference, have demonstrated that hypericin, a natural product found in plants, inhibits the replication of Friend leukemia virus and radiation leukemia virus, both in vitro and in vivo. Meruelo et al., also stated that when hypericin was administered to mice at doses sufficient to prevent retroviral-induced disease, the mice appeared to be devoid of undesirable side effects. Meruelo et al. have reported that hypericin can reduce the spread of HIV in vitro. Meruelo et al. speculate that hypericin may act by

direct inactivation of the virions. Inactivation of the virus particles is considered likely because adding hypericin to the growth medium of infected cultures, just before the harvest of the virus, causes substantially diminished reverse transcriptase (RT) activity in the culture supernatants and hypericin does not directly inhibit purified RT.

Chanh et al., "Photodynamic Inactivation of Simian Immunodeficiency Virus," J. of Virological Methods, Vol. 26, pp. 125-132 (1989), which is incorporated herein in its entirety by reference, disclose photodynamic inactivation of simian immunodeficiency virus (SIV). A dihematoporphyrin ether (DHE) was used to inactivate, in vitro, the infectivity of SIV. DHE was activated through the use of a laser beam. The experiment was conducted by incubating SIV suspended in a culture medium with DHE in the dark, followed by irradiation in the flow cell. Cells were washed and fed into a culture medium and then stained. The authors postulate that this treatment will be viable in reducing the risk of infections by enveloped viral infectious agents which may occur during transfusion.

Lavie et al., "Studies of Mechanisms of Action of the Antiretroviral Agents Hypericin and Pseudohypericin," Proc. Natl. Acad. Sci., Vol. 86, pp. 5963-5967, (August

1989), which is incorporated herein in its entirety by reference, disclose that hypericin and pseudohypericin possess anti-retroviral activity. Specifically, the hypericin and pseudohypericin suppress the spread of murine retrovirus in vivo and in vitro. Treatment of hypericin and pseudohypericin results in complete inactivation of reverse transcriptase of both murine and human viruses. The compounds were administered in the tested animals by way of injection.

Hudson et al., "Antiviral Activities of Hypericin," Antiviral Research, Vol. 15, pp. 101-112 (1991), which is incorporated herein in its entirety by reference, disclose that hypericin inactivates murine cytomegalovirus (MCMV) Sindbis Virus, and HIV-1. The antiviral effect of hypericin was augmented by visible light.

United States Patents numbers 4,898,891, to Lavie et al, 5,049,589 to Lavie et al and 5,047,435 to Lavie et al, each of which is incorporated herein by reference, disclose the antiviral effects of hypericin and pseudohypericin. The '891 patent discloses an antiviral pharmaceutical composition containing hypericin as an active ingredient and the use of the composition in treating viral infections. The '435 patent also discloses a method for treating viral infections using hypericin. The '589

patent discloses an antiviral aerosol pharmaceutical composition containing hypericin.

Finally, Matthews et al., "Photodynamic Therapy of Viral Contaminants with Potential for Blood Banking Applications," Transfusion, Vol. 28, No. 1, pp. 81-83 (1988), which is incorporated herein in its entirety by reference, disclose photodynamic therapy for eradicating viral contaminants. The photodynamic method disclosed employs a hematoporphyrin derivative used as the photosensitizer, which, in a static fluid system, photoinactivates an enveloped virus. Hematoporphyrin is a porphyrin compound which is known for its affinity for tumor cells. Thus, the hematoporphyrin derivative can be used to destroy tumor cells and viruses selectively. The method of Matthews et al. involves extracorporeal plasmaphoresis, i.e., the blood is taken out of the body prior to being treated with hematoporphyrin and light. The method disclosed in Matthews et al. uses visible light as its outside light source.

There is therefore a need to connect an energy source to the photoactive chemicals so that photodynamic therapy can be expanded to all regions of the body. There is also a need to provide a method for using photodynamic

therapy only in the cells infected by a virus or only in tumor cells. Thus, activation of the energy source must be regulated such that activation preferentially occurs in the virus-infected cells or tumor cells.

The present invention overcomes the problems in the prior art by employing an energy source which emits energy in a broad band of wavelengths in the range where the photoactive chemical absorbs. The energy source is chemically activated by another chemical, which is regulated to express only in the virus-infected or tumor cell. Thus, photoactivation of the photosensitizer occurs only in virus-infected cell or tumor cells.

The efficiency of the system depends on the energy transfer of the system which depends on the distance and orientation between the donor and acceptor, i.e., the energy source and the photoactive chemical. Accordingly, the inventors also developed a chemical tether to connect the energy source and the photoactive chemical. The use of such a tethered compound allows for the in vivo introduction of an internal chemically-activated light source having broad applications in antiviral and tumor therapy.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an antiviral composition.

It is a further object of the invention to provide a tethered compound for use in the antiviral composition.

Another object of the invention is to provide an antitumor composition.

Another object of the invention is to provide a tethered compound for use in the antitumor composition.

Another object of the invention is to provide a hypericin analog for use as an intermediate in the preparation of the tethered compounds.

Another object of the invention is to provide luciferin and its analogs for use as an intermediate in the preparation of the tethered compounds.

Yet another object of the invention is to provide a method for synthesizing precursors of the luciferin analogs.

Yet another object of the invention is to provide a method for synthesizing the tethered compounds.

A still further object of the invention is to provide means for activating the tethered compounds.

A still further object of the invention is to provide an expression plasmid for activating the tethered compounds.

A still further object of the invention is to provide a liposome containing the expression plasmid.

A still further object of the invention is to provide a transfected cell containing the expression plasmid.

A still further object of the invention is to provide an eukaryotic cell containing a stably integrated copy of the expression plasmid.

A still further object of the invention is to provide a viral vector produced from the eukaryotic cell.

A still further object of the invention is a method for inactivating a virus.

A still further object of the invention is to provide a method for destroying tumor cells.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by the practice of the invention. The objects and advantages of the invention will be attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

To achieve the objects in accordance with the purpose of the invention as embodied and broadly described herein, the present invention provides for a composition for inactivating a virus or destroying a tumor cell. The composition preferably contains three components. The first component is a chemical capable of photosensitization, hereinafter termed "a photosensitizing chemical." The second component is an energy donating chemical. The third component contains chemical means for activating the transfer of energy or the emission of light from the energy donating chemical. The present invention preferably employs luciferin, a natural light source, or

its analogs as the energy donating chemical. The photosensitizing chemical is preferably hypericin, a porphyrin, or one of their analogs.

In a preferred antiviral composition, as well as in a preferred antitumor composition, the hypericin, porphyrin, or analog is connected to the luciferin or analog, preferably by way of a chemical tether or chemical linker. Thus, in a preferred embodiment of the invention, the first and second components of the antiviral or antitumor composition, i.e., the photosensitizing chemical and the energy emitting chemical are connected by a chemical tether and form a tethered compound. As used herein, "chemical tether" or "chemical linker" is a chemical connector of two ring compounds. The nature of the connection between hypericin and luciferin, a preferred tethered compound, may affect either the efficiency of energy transfer or the ability of hypericin to interact within the cell membrane.

The hypericin-luciferin tethered compound is preferably prepared through a condensation reaction of an activated hypericin with a luciferin analog. The luciferin analogs are synthesized from known compounds.

The antiviral and antitumor compositions of the present invention also contain a third component that contains chemical means for activating the transfer of energy or emission of light from the energy donating chemical. The chemical means of the present invention is an activating chemical encoded by a gene under control of regulatory genetic elements. The DNA controlling the activating chemical operably contains regulatory motifs recognized by host cell transcription factors in addition to motifs recognized by viral regulatory proteins. As used herein, "regulatory element" ("regulatory nucleic acid sequence") is a noncoding region (sequence) that determines when, if, and at what level the DNA encoding the activating chemical is expressed. Such regulatory elements include promoters, enhancers, and transcription and translation initiation and termination sequences. As used herein, "nucleic acid sequence" refers generally to a polynucleotide molecule, more specifically to a linear series of nucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of the two adjacent pentoses.

Luciferin, a preferred energy donating molecule of the present invention, transfers energy or emits light when it reacts with the enzyme luciferase, ATP, and molecular oxygen, i.e., when the luciferase activates luciferin.

Therefore, luciferase is an activating chemical when luciferin is the energy donating chemical. Thus, another preferred embodiment of the invention is regulating the expression of luciferase such that luciferase is only expressed in virus-infected or tumor cells.

Regulating the activating chemical of an antiviral composition is preferably accomplished by first constructing an expression plasmid containing a gene encoding the activating chemical under control of a promoter. The DNA encoding the activating chemical is inserted into a vector, such as an expression plasmid, in proper orientation and correct reading frame for expression. Preferably, the antiviral composition is used to inactivate enveloped virus but is useful for inactivating any viruses. More preferably, the antiviral composition is used to inactivate DNA enveloped viruses such as Herpes Simplex Virus and RNA enveloped viruses such as lentiviruses HIV and EIAV. The expression plasmid preferably contains a luciferase gene under control of an enveloped virus promoter, e.g., the retrovirus long terminal repeat. The promoter is selected such that replication of the virus will transactivate the promoter resulting in increased expression of luciferase leading to activation of luciferin and photoactivation of hypericin. Thus, photoactivation is localized to the virus-infected

cells, thereby targeting the antiviral activity of the photosensitizing chemical.

The expression plasmid may be introduced into a cell by a variety of known methods including incorporation into a liposome. Other known methods that may be employed include, but are not limited to, the use of naked DNA transfer, microinjection, or calcium phosphate precipitation. For ultimate application in treatment, it may be necessary to construct a viral vector that is introduced into a cell by viral mediated gene therapy or is introduced into a cell by other known gene therapy techniques. Such a viral vector is constructed by stably integrating a copy of the expression plasmid into a cell line. The viral vector is then produced from the cell line.

If the tethered compound is used in an antitumor composition, the regulation of the activating chemical is preferably accomplished by constructing an expression plasmid. The expression plasmid contains the gene encoding the activating chemical e.g., luciferase, in an embodiment when luciferin is the energy donating chemical, under control of a promoter. The promoter is transactivated by elevated levels of proteins expressed in tumor cells. Transactivating the promoter increases expression of

luciferase which activates luciferin and photoactivates the photosensitizing chemical, i.e., hypericin in one embodiment. Thus, photoactivation is localized to the tumor cell, thereby targeting the antitumor activity of the photosensitizing chemical.

The expression plasmid used for regulating the activating chemical of the antitumor composition, may be introduced into a cell by methods similar to those discussed for the antiviral composition. These methods include, but are not limited to, incorporation into a liposome, naked DNA transfer, microinjection, or calcium phosphate precipitation. For ultimate application in treatment, it may be necessary to construct a viral vector that is introduced into a cell through known gene therapy techniques. Such a viral vector is constructed by stably integrating a copy of the expression plasmid into a cell line. The viral vector is then produced from the cell line.

The tethered compounds of the present invention are synthesized through a condensation reaction of the energy donating chemical with the photosensitizing chemical. In preferred embodiments of the present invention, the tethered compounds are synthesized by condensing a

porphyrin, hypericin or their analogs with luciferin or its analogs.

The antiviral composition containing tethered compounds of the present invention may be used in a method for inactivating a virus. Although the antiviral compositions may be used to inactivate any virus, the antiviral compositions are preferably used to inactivate either DNA or RNA enveloped viruses. In a preferred method, the first and second components of the antiviral composition, preferably in the form of a tethered compound, are administered in pharmaceutically effective amount to the virus-infected cell. In addition, the third component of the antiviral composition, i.e., the component containing the chemical means for activating the transfer of energy or the emission of light, is also administered to the virus-infected cell, as discussed above, where it then activates the energy donating chemical which activates the photosensitizing chemical which inactivates the virus.

The antitumor composition containing tethered compounds of the present invention may also be used in a method for treating tumors by destroying neoplastic cells. In a preferred method, the first and second components of the antitumor composition, preferably in the form of a tethered compound, are administered in a pharmaceutically

effective amount to a neoplastic cell. In addition, the third component of the antitumor component, i.e., the component containing the chemical means for activating the transfer of energy or the emission of light, is also administered to the neoplastic cell, as discussed above, where it then activates the energy donating chemical which then activates the photosensitizing chemical which destroys the neoplastic cell.

DESCRIPTION OF THE FIGURES

Figure 1 is a graphic representation of the comparison of the spectrum of the chemiluminescent emission of the luciferase-catalyzed oxidation of luciferin and the absorption spectrum of hypericin in the red region of the visible spectrum.

Figure 2 is a graphic representation of the time course of the chemiluminescent emission from the luciferase-catalyzed oxidation of luciferin. The concentration of the reactants are as follows: [luciferase] = 2.67×10^{-7}M, [luciferin] = 1.18×10^{-6}M, [ATP] = 5×10^{-5}M; the buffer is 25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 15 mM K_3PO_4 at pH = 7.75 and the reaction is carried out at 25° C.

Figure 3 is a representation of an expression plasmid containing the luciferase gene under the control of the EIAV LTR.

Figure 4 shows the antiviral activity of hypericin in the presence of luciferin and luciferase.

Figure 5 shows the expression of luciferase in ED cells in the presence of EIAV or ETat

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to the presently preferred embodiments of the invention, which together with the following examples, serve to explain the principles of the invention.

The invention relates to a composition for inactivation of a virus comprising a first component containing photosensitizing chemical, a second component containing an energy donating chemical, and a third component containing means for regulating the emission of light or transfer of energy as well as, to methods for synthesizing such components. The energy donating molecule should emit energy or light in a broad band of wavelengths in the range where the photoactive chemical absorbs. As

used herein, a photosensitizing chemical is a chemical that is activated by light or energy transfer.

A preferable natural energy source is luciferin and its analogs. The reaction of luciferin with the enzyme luciferase, ATP, and molecular oxygen produces an intense long-lived emission from a triplet state, phosphorescence. The light produced by luciferin and its analogs is in the 520-680 nm region. Luciferin does not emit light or energy unless activated by luciferase. Thus, regulating the expression of luciferase regulates the activation of luciferin.

The photosensitizing chemical is preferably one selected from hypericin, other quinones, hematoporphyrin derivatives, phthalocyanins and porphyrins. A specific embodiment involves the use of hypericin as the photosensitizing chemical. Hypericin absorbs light in the 540-660 nm range. When hypericin is photoactivated, it produces singlet oxygen with a quantum yield of 0.74.

In one embodiment, hypericin and luciferin/luciferase (commercially available) are aerobically mixed in the dark in the presence of a virus. In one case, the virus is a lentivirus, specifically, equine infectious anemia virus (EIAV). The luciferase

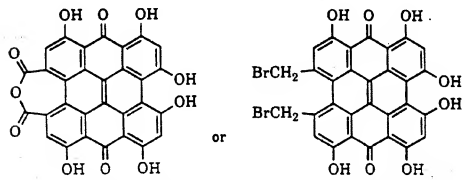
activates the luciferin which emits light and photoactivates the hypericin and reduces the infectivity of EIAV. The efficiency of the transfer of energy from luciferin to hypericin depends on the distance and orientation between the energy donating chemical and the acceptor, i.e. the photosensitizing chemical.

The energy transfer efficiency is optimized by connecting the energy donating chemical to a photosensitizing chemical by way of a chemical tether or chemical linker. The chemical tether or linker connects the two ring compounds. Thus, a preferred embodiment of the invention includes the first and second components in the form of a tethered compound.

The nature of the connection between the photosensitizing chemical and the energy donating chemical may affect either the efficiency of the energy transfer -- conformational constraints -- or the ability of the photosensitizing chemical to interact with the cell membrane. The tethering of the photosensitizing chemical and the energy donating chemical is directed to finding the most efficient energy transfer between the donor and the acceptor. The most efficient transfer will depend on the relative separation and orientation of each of the two components.

Specifically, a preferred embodiment is directed to the connection between hypericin and luciferin or its analogs. Hypericin is a preferred photosensitizing chemical because it is already known that low dosage administration of hypericin has been shown to avoid the undesirable side effects. See Meruelo et al., Proc. Natl. Acad. Sci., Vol. 85, pp. 5230-5234, July 1988, incorporated herein in its entirety by reference.

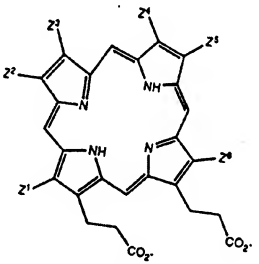
A preferred embodiment of the invention is directed to the use of an activated hypericin analog, an anhyride or halide, for use as an intermediate in the preparation of the tethered compound. The anhyride and halide have the following formula:



The anhyride can be prepared in only three steps from hypericin. Acetylation of hypericin followed by chromic acid oxidation generates the diacid, which when

reacted with DCC completes the synthesis of the anhydride. The halide is made by acetylation followed by benzylic halogenation.

Another embodiment of the invention is directed to using, as the photosensitizing chemical, porphyrins of the following formula:

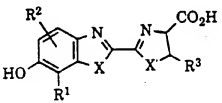


wherein Z^1 - Z^6 are alkyl or alkenyl groups having 1 - 15 carbon atoms.

Luciferin and its analogs can be used to form tethered compounds with photoactive chemicals such as phorphyrins, hypericin, or other quinones. The tethered compounds must demonstrate overlap in the absorption spectra of the selected photoactive chemical with the emissions spectra of the energy donating chemical.

When oxidized, luciferin's broad emission band is centered at 560 nm and overlaps the two strongly absorbing bands of hypericin in the red region of the visible spectrum at 555 and 600 nm. See Figure 1 for a comparison of the spectrum of the chemiluminescent emission of the luciferase-catalyzed oxidation of luciferin and the absorption spectrum of hypericin in the red region of the visible spectrum. This overlap allows for luciferin to be paired with hypericin.

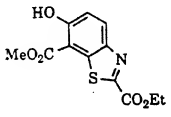
During the luciferase-catalyzed oxidation of luciferin, one photon of light is produced per molecule of substrate consumed. Figure 2 presents the time course of the chemiluminesecent reaction of luciferin. The tethering of luciferin or its analogs must not disrupt the recognition and binding of the luciferin substrate by the enzyme for the light producing reaction, luciferase. Therefore, the preferred luciferin analogs for use in preparing the tethered compound are of the following formula:



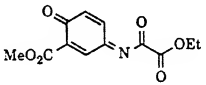
The tether or chemical linker is located at either R_1, R_2, R_3 or R_4. X and X' may be one of the following: S, O, H,H, CH=CH or NR_4. When R_1 is the site of the tether, R_1 is $-CO_2(CH_2)_nY$, wherein n is 2-15; Y is OH, SH or NH_2; and R_2-R_4 are H. When R_2 is the site of the tether, R_2 can be one of the following: $-(CH_2)_nCO_2H$ and $-S(CH_2)_nY$, wherein n is 1-15; Y is OH, SH or NH_2; and R_1 and R_3-R_4 are H. When R_3 is the site of the tether, R_3 can be one of the following: $-CO_2H$ and $-(CH_2)_nY$, wherein n is 1-15; Y is OH, SH or NH_2; and R_1-R_2 and R_4 are H. When R_4 is the site of the tether, R_4 is $-(CH_2)_nY$, wherein n is 2-15; Y is OH, SH or NH_2; and R_1-R_3 are H. The formula above displays luciferin when R_1-R_3 are H and both X and X' are both S.

Luciferin analogs may be synthesized in a number of ways, depending on which analog is to be used.

First, a benzothiazole intermediate is employed in the formation of a luciferin analog of the above-formula. The benzothiazole employed has the following formula:

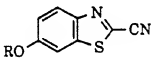


The benzothiazole is synthesized by adding hydrogen sulfide to an electron deficient imino quinone of the following formula:



The resulting adduct undergoes dehydration to form the luciferin analog.

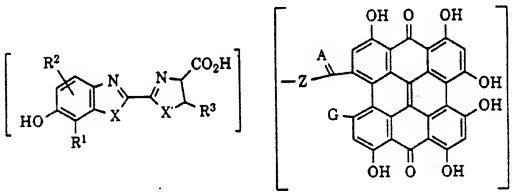
A second intermediate, a benzothiazole hydroxy nitrile has the following formula:



R is Me, H or $PhCH_2$. The benzothiazole hydroxy nitrile is formed by adding cyanide ion to a chloroalkoxybenzothiazole followed by dealkylation to form the benzothiazole nitrile.

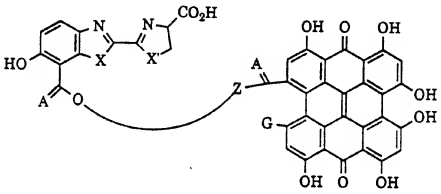
Luciferin analogs are then synthesized by adding cysteine or a substituted cysteine to either one of the intermediates synthesized above.

Once the luciferin analogs are synthesized, these compounds are then reacted in a condensation reaction with the activated hypericin to form the tethered molecule of the following formula:



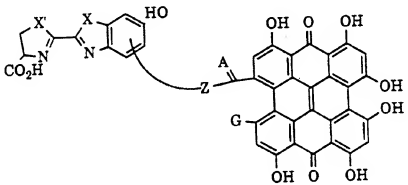
X and X' may be one of the following: S, O, H,H, CH=CH or NR_4. The tether or chemical linker of the tethered molecule occurs in one of four locations on the luciferin analog: R_1, R_2, R_3, or R_4. When the tether is located at

R_1, the tethered compound has the following formula:



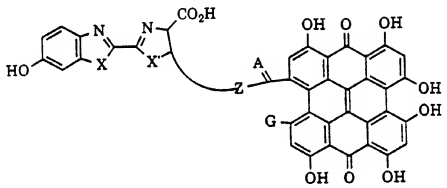
R_1 is $-CO_2(CH_2)_nZ$, wherein n is 2 to 15; and R_2-R_4 are H; Z is O, S or NH; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

If R_2 is the site of the tether, then the compound has the following formula:



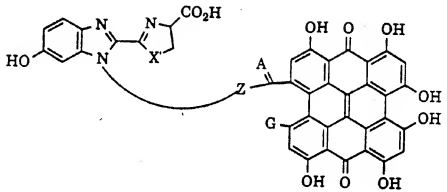
R_2 may be one of the following: $-(CH_2)_nCOZ$ and $-S(CH_2)_{n+1}Z$, wherein n is 1-15; R_1, R_3 and R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, CH_2Br.

When R_3 is the site for the tether, the compound has the following formula:



R_3 may be one of the following: -COZ and $-(CH_2)_nZ$, wherein n is 1-15; R_1-R_2 and R_4 are H; Z is O, NH, or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, CH_2Br.

When R_4 is the site of the tether, the compound has the following formula:

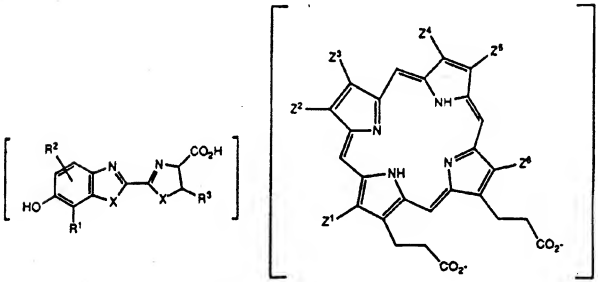


R_4 is $-(CH_2)_nZ$, wherein n is 2-15; R_1-R_3 are H; Z is O, NH, or S; A is O, or H,H; and G is CH_3, CO_2H, CO_2Me, CH_2Br.

The tethered compound may also contain the luciferin analogs discussed above linked to the porphyrin compound discussed above. As in the luciferin-hypericin tethered compound, the site of the tether or chemical linker, may be at R_1, R_2, R_3 or R_4. The tether is attached to the porphyrin at either its two carbonyls. The luciferin-porphyrin tethered compounds will be similar in structure to luciferin-hypericin tethered compounds except for the substitution of porphyrin for hypericin, i.e., the

luciferin and tether or linker portion remain the same as those described above.

When the tethered compound contains a porphyrin instead of hypericin the compound has the following general formula:



The tether is attached via R_1, R_2, R_3 or X (through NR_4) as explained and defined above. Z^1 - Z^6 are each alkyl or alkenyl groups containing 1-15 carbon atoms.

The invention further relates to a composition for destroying tumor cells comprising a first component containing a photosensitizing chemical, as defined above, a second component containing an energy donating chemical, and a third component containing means for regulating the emission of light or transfer of energy, as well as to methods for synthesizing such components. The energy donating molecule should emit energy or light in a broad band of wavelenghs in the range where the photosensitizing chemical absorbs.

The antitumor composition preferably contains luciferin or an analog as described above, as the energy donating chemical and a porphyrin, hypericin or an analog as the photosensitizing chemical. As explained above, the energy donating chemical and the photosensitizing chemical are optionally connected to each other by way of a chemical tether or chemical linker as defined above. The tethered compound used in a preferred antitumor composition contains (1) luciferin analog and (2) hypericin or a porphyrin and is synthesized as previously described.

The tethered compounds of the present invention can contain any combination of photosensitizing chemicals and energy donating chemicals linked by a tether, providing that the energy donating molecule activates the

photosensitizing molecule in sufficient degree to maintain the antiviral or antitumor activity of the photosensitizing molecule. Tether selection is based on (1) the rate of transfer from the donor to acceptor and (2) the recognition of the substrate by the catalyst for the light producing reaction. The rate and quantum yield of the enzyme catalyzed reaction must be monitored in order to screen for the potential effects of inhibition of reactivity.

When used for treating virus infections such as DNA or RNA enveloped virus infections, the first and second components of the antiviral composition, preferably in the form of a tethered compound, may be administered orally, parenterally, and preferably intravenously. In any event, a pharmaceutically effective amount of the compound is administered. An effective amount is determinable by persons skilled in the art in the view of teachings disclosed herein.

Further, the first and second components, preferably in the form of a tethered compounds, can be used at dosages containing from about 0.001 micrograms to about 100,000 micrograms per kilogram body weight per treatment, preferably between about 1 microgram and about 5×10^4 micrograms per kilogram of body weight per treatment.

The duration and number of doses or treatments required to control a particular virus will vary from subject to subject, depending upon the severity and stage of the illness and the subject's general condition and will also depend on the activating chemical, as well as the toxicity (if any) of the tethered compound. This will be determinable by persons skilled in the art in view of the teachings contained herein. The total dose required for each treatment may be administered in divided doses or in a single dose. The preferred form of the first and second components, i.e., the tethered compound, may be administered daily, more than once daily, one or two times a week, or as determined by the subject's condition and the stage of the disease.

Those skilled in the art will appreciate that the frequency of treatment is subject to optimization, which can be determined by routine experimentation according to methods well known in the art, e.g. by establishing a matrix of dosage and frequency and assigning a group of experimental subjects to each point of the matrix. Design of this experiment should preferably also take into account the tissue accumulation properties of the compounds of the present invention.

The present invention also provides pharmaceutical compositions and formulations for treating lentiviral infections. The first and second components, preferably in the form of a tethered compound, can be incorporated in conventional, solid and liquid pharmaceutical formulations (e.g. tablets, capsules, caplets, injectable and orally administrable solutions) for use in treating mammals that are afflicted with viral infections. The pharmaceutical formulations of the invention comprise an effective amount of the tethered compounds of the present invention (as disclosed above) as the active ingredients. For example, a parenteral therapeutic composition may comprise a sterile isotonic saline solution containing between about 0.001 micrograms and about 100,000 micrograms of the tethered compounds of the present invention as described above. It will be appreciated that the unit content of active ingredients contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of capsules, tablets, injections or combinations thereof.

Each formulation according to the present invention may additionally comprise inert constituents including pharmaceutically-acceptable carriers, diluents, fillers,

salts, and other materials well-known in the art the selection of which depends upon the dosage form utilized and the particular purpose to be achieved according to the determination of the ordinarily skilled artisan in the field. For example, tablets may be formulated in accordance with conventional procedures employing solid carriers well known in the art. Examples of solid carriers include, starch, sugar, bentonite, silica and other commonly used carriers. Propylene glycol, benzyl alcohol, isopropanol, ethanol, dimethylsulfoxide (DMSO) dimethylacetamide or other biologically acceptable organic solvents or aqueous solutions (e.g. water with a pH higher than 7 and preferably about 8) may be used as diluents, carriers or solvents in the preparation of solid and liquid pharmaceutical formulations containing the anti-lentiviral compositions of the present invention. Further nonlimiting examples of carriers and diluents include carbohydrates, albumin and/or other plasma protein components such as low density lipoproteins, high density lipoproteins and the lipids with which these serum proteins are associated. Such lipids include phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine and neutral lipids such as triglycerides. Additional lipid carriers include without limitation tocopherol, retinoic acid and cyclodextranes. Semisolid formulations such as those

well-known in the art (e.g. suppositories) are also contemplated.

Preferred parenteral dosage forms may comprise, for example, an isotonic saline solution, containing between about 0.1 micrograms to about 100,000 micrograms of the tethered compounds of the present invention.

Capsules employed in the present invention may be made from any pharmaceutically acceptable material, such as gelatin or cellulose derivatives. Sustained release oral and transdermal delivery systems are also contemplated, as is interveneous injection.

The antiviral composition containing the preferred luciferin-hypericin tethered molecule requires luciferase for the catalysis of the light producing reaction and the activation of hypericin. The activation of the light source is specifically regulated such that the hypericin is photoactivated only where needed. The regulation of luciferin activation is achieved by regulating the expression of luciferase.

One embodiment of this invention is directed to regulating the expression of the activating chemical of an antiviral composition. The third component of the

antiviral composition, i.e., the component containing the chemical means for activating the transfer of energy, is responsible for regulating the expression of the activating chemical. In a preferred antiviral composition, the activating chemical is luciferase. Luciferase is regulated by placing the gene encoding luciferase under control of a promoter that is transactivated by replication of the virus. By placing the expression of luciferase under control of such a promoter, replication of the virus transactivates the viral promoter resulting in an increased expression of luciferase leading to an activation of luciferin and the photoactivation of hypericin. The events are localized in virus-infected cells thereby targeting the antiviral activity of hypericin.

Specifically, the expression of luciferase is regulated to the virus-infected cells by constructing an expression plasmid which contains the gene coding for luciferase under control of a promoter that is transactivated by repliction of said virus. Example of such promoters include HIV TAR, the sequence of which is incorporated herein by reference, and which is described in Berkhout et al., Cell, 59: 273-282, (1989); Berkhout et al., Cell, 62: 757-767 (1990) and Berkhout et al., J. Virol., 66: 139-149 (1992), each of which are incorporated herein by reference, or the consensus enhancer sequence

present in the promoter of Herpes Simplex Virus alpha genes, the sequence of which is incorporated herein by reference, and which is described in Fields et al., Virology, Second Ed. 1990, and Mackem et al. J. Virol, 44: 939-949, (1982) each of which is incorporated herein by reference, and the EIAV long terminal repeat, the sequence of which is incorporated herein by reference, and which is shown in Figure 1 of Carpenter et al., J. Virol., 65(3); 1605-1610 (1991), which is incorporated herein by reference.

Figure 3 shows a representation of the expression plasmid containing the luciferase gene under control of the EIAV LTR. The plasmid containing the EIAV LTR is transfected into equine dermal cells (ED) and there expresses luciferase in the presence of either EIAV or the viral transactivating protein, Tat, but not in normal ED cells. In one embodiment, the plasmid is first placed into a liposome before it is directly transfected into equine dermal cells.

Any method of introducing DNA into a cell is sufficient for the gene transfer and therapy herein described. Known methods for transferring DNA into cells include the use of viral vectors, microinjection, liposome mediated, calcium phosphate precipitation and simple naked DNA

transfer. See Lim et al., Molec. and Cel. Bio., 7(10): 3459-3465 (1987); Kasid et al., PNAS, 87: 473-477 (1990); Gilboa, Eli, Retrovirus and Disease, Academic Press, Inc. pgs. 95-111 (1989); Kantoff et al, PNAS 83: 6563-6567 (1986); Kasid et al., PNAS, 87: 473-477 (1990); Kantoff et al., J. Exp. Med, 166: 219-234 (1987); Cornetta et al., J. Virol. Meth., 23: 187-194 (1989) and Culver et al., PNAS, 88: 3155-3159 (1991). The disclosure of each of these articles are incorporated herein by reference.

The third component of the antiviral composition, i.e., the component containing the means for activating the transfer of energy is preferably introduced into patients through gene therapy techniques. In one embodiment, the therapy involves retroviral mediated gene therapy that includes the ultimate construction of a retroviral vector in which the DNA encoding the activating chemical, e.g. luciferase, is placed under the control of a modified retroviral promoter which is activated in virus-infected cells. In one embodiment, the therapy involves first constructing a plasmid vector containing a retroviral LTR, retroviral packaging sequences, and the DNA encoding luciferase. Additionally, the vector may contain DNA encoding a selectable marker, e.g. neomycin resistance.

The plasmid is introduced into a eukaryotic cell or cell line, preferably a packaging cell, which harbors stably integrated proviral sequences sufficient for expression of retroviral structural proteins, but which are deficient in sequences required for packaging and replication of RNA transcribed by proviral DNA. Following introduction of the plasmid vector, cells containing stably integrated plasmid DNA are identified by expression of the selectable marker. Encapsidation of vector sequences by the proviral structural proteins results in production of retrovirus particles which contain genetic material encoding the activating chemical under the control of a regulated promoter. Transfected cells which produce such retrovirus particles are referred to as producer cells, and the virus particle produced from these cells is referred to as the retrovirus vector.

The virus vector may be introduced into human and mammalian cells in a variety of methods. One method comprises removing cells, e.g., lymph cells from the patients infected with the virus to be inactivated. The removed cells are then infected with the constructed viral vectors or by other means of introducing DNA such as liposome mediated transfer. If the DNA is introduced by liposome mediated transfer, the liposome contains plasmid DNA encoding the promoter controlling expression of the

activating chemical, the activating chemical and in some embodiments, a selectable marker. The patient cells containing the gene encoding the activating chemical are selected through the use of the selectable marker, i.e., cells are selected if they demonstrate neomycin resistance. The selected cells are then grown and reintroduced into the patient.

Alternatively, the producer cell lines, constructed as described above, are introduced directly into the patient, resulting in infection in vivo. Further, it is possible to introduce the virus vectors directly into the patient.

In each of the above-described embodiments, the DNA encoding the activating chemical is stably integrated within the patient's cell. Expression of the activating chemical is regulated, such that only virus-infected cells express high levels of the activating chemical. The frequency of administering the third component via gene therapy or other known techniques will depend on how long the inserted DNA can be expressed and how long the cells containing the inserted DNA will survive. The first and second components, preferably in the form of a tethered compound, must be administered by means and in dosages as described above. In cells expressing high levels of the

activating chemical, i.e., virus-infected cells, the activation of the energy donating chemical activates the photosensitizing chemical, which in turn inactivates the virus.

In one specific embodiment of the invention, the tethered compound is used to inactivate HIV, a RNA lentivirus. The tethered compound comprises hypericin and a luciferin analog, the first and second components, and is administered as discussed herein. The third component containing luciferase is administered by constructing a viral vector containing (1) a promoter that contains HIV TAR and NF-kB and SP-1 sites upstream or sequences necessary for TAT-mediated transactivation, (2) the packaging sequences, (3) the luciferase gene and (4) Neo^r, a neomycin resistance marker. The packaging cell line includes a provirus expressing HIV env/gp 120 to target $CD4^+$ cells, i.e., cells in which HIV replicates, and no packaging sequence. The resultant virus vector line is infected into patient cells either in vivo or ex vivo. Once the genetic material is integrated into the patient's cells, luciferase is expressed to high levels only in HIV infected cells. The expressed luciferase activates the luciferin analog of the administered tethered compound. The luciferin analog in turn activates the photosensitizing chemical, of the administered tethered compound. The

photosensitizing chemical, once activated by the luciferin analog, inactivates HIV.

The means for regulating the activating chemical which activates the energy donating chemical, i.e., in the preferred embodiment, luciferase and luciferin, are not the same when the composition is used to destroy tumor cells as those in an antiviral composition.

Specifically, the third component of an antitumor composition containing DNA encoding an activating chemical is located on an expression plasmid. The DNA is under control of a different promoter, such as the carcino-embryonic-antigen (CEA) promoter. Increased expression of the activating chemical occurs when levels of certain proteins e.g., CEA protein, are elevated. Thus, the promoter is activated by levels of certain proteins that are elevated only in tumor cells. Therefore, the expression of the activating chemical, i.e., luciferase, are localized only to tumor cells.

The gene transfer into tumor cells occurs through methods as those described herein for transfer into virus-infected cells; i.e., via the use of known methods such as viral vectors, liposome mediated transfer, microinjection and naked DNA transfer.

The compositions containing the photosensitizing chemicals and the energy donating chemicals, optionally connected by a tether, and the chemical activating means are used to inactivate a virus or destroy tumor cells. The compositions maybe used to inactivate any virus, preferably DNA and RNA enveloped viruses. An example of a preferred DNA enveloped virus is Herpes Simplex Virus. Examples of RNA enveloped viruses include lentiviruses EIAV and HIV. Inactivation of a virus occurs when the photosensitizing chemical (component one) and the energy donating chemical (component two), preferably in the form of a tethered compound, are introduced into the virus-infected cell along with the means for activating the energy donating chemical (component three). As discussed above, the preferred embodiment provides that replication of the virus transactivates the promoter, which increases expression of luciferase, which activates luciferin, which photoactivates hypericin, resulting in the inactivation of the virus. However, the expression of the activating chemical may be regulated by alternative means that increase expression of the activating chemical in the virus-infected cells.

The invention also provides a method for destroying neoplastic cells, preferably malignant cells. Thus, it can be used to treat tumors preferably careers.

The method for destroying a neoplastic cell is similar to that of inactivating a virus in that the photosensitizing chemical (component one) and the energy donating chemical (component two), preferably in the form of a tethered compound, are introduced into the tumor cell along with the means for activating the energy donating chemical (component three). However, in this preferred embodiment, elevated levels of certain proteins present in tumor cells increases expression of the activating chemical, which then activates the energy donating chemical, which photoactivates the photosensitizing chemical, resulting in destruction of the tumor cell. Again, expression of the activating chemical may be regulated by any available means.

It is to be understood that the application of the teachings or the present invention to a specific problem or environment will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the compounds and compositions of the present invention and methods of their preparation and for their use appear in the following examples.

Examples of the preferred embodiments of the present invention appear in the following examples.

EXAMPLE 1

Testing Of An Antiviral Composition Containing Hypericin And Luciferin

Initially, hypericin and luciferin/luciferase (commercially available) were mixed under aerobic conditions in the dark in the presence of EIAV according to the following protocol:

Materials and Methods

Serial ten-fold dilutions of EIAV in Hank's balanced salt solution were mixed with an equal volume of luciferase assay buffer containing 25 mM glycylglycine, pH 7.8, 15 mM $MgSO_4$, 4 mM EGTA, 150 mM KPO_4, 2 mM ATP, and 1 mM DTT. Luciferin and luciferase were added to final concentrations of 0.4 mM and $1.6X10^{-7}$ M, respectively. The reactions were incubated in the dark for 45 min, and ten-fold serial dilutions were inoculated onto 10^6 ED cells in the presence of polybrene. Cells were incubated 5 days at 37°C, fixed in 100% methanol and stained for the presence of EIAV as we previously described Carpenter et al., J. Virol, 65(3): 1605-1610 (1991), incorporated in its

entirety herein by reference. Foci of EIAV-infected cells were quantitated and the results expressed as focus-forming units per ml (FFU/ML).

Results

Results of these experiments indicated that the hypericin activated by chemiluminescence was dependent on the concentration of hypericin (Figure 4). In addition, the antiviral activity of hypericin was only 1-5% as efficient as what we previously observed using white light Carpenter and Kraus, Photochem and Photobio., 53(2): 169-174 (1991) incorporated in its entirety herein by reference. The results demonstrate that the luciferase catalyzed luciferin reaction photoactivates hypericin in vitro.

EXAMPLE 2

Synthesis Of A Hypericin Analog
For Use In Preparing A Tethered Compound

To a stirred suspension of the diacid formed by acetylation of hypericin followed by chromic acid oxidation, (H.J. Banks et al. Aust. J. Chem., 1976,29,1509) in ether-chloroform at 0°C was added dicyclohexylcarbodiimide

(1.3 eq). The suspension was allowed to slowly warm to rt over one day. A precipitate was filtered. The filtrate was concentrated to give the anhydride (unstable) which was immediately reacted with the luciferin analogs synthesized as described below.

EXAMPLES 3-4

Synthesis Of Precursors For
Luciferin And Its Analogs

Precursor A - Benzothiazole

To a stirred solution of the methyl ester of 2-hydroxy-5-aminobenzoic acid was added ethylchlorooxalate (1.2 eq) in and pyridine (1.3 eq) in methylene chloride (1 M) at 0°C. After stirring overnight, the solvent was removed and the residue was chromatographed using hexanes:ethyl acetate.

The diester amide (1 eq) was dissolved in acetic acid (1 M). To this solution was added 1.2 eq of lead tetraacetate. A precipitate which developed was filtered, washed and taken on to the next step.

The iminoquinone (1 eq) was dissolved in pyridine (1 M) and reacted with excess hydrogen sulfide at 0°C.

After warming to rt overnight, the solution was heated to 50°C, cooled and concentrated in vacuo. The residue was purified by chromatography using ethyl acetate:methylene chloride to afford the benzothiazole in 45% yield over two steps. 2: NMR ($CDCl_3$): 1.17(t, J=7 Hz, 3H), 3.75(s, 3H), 4.25(q, J=7 Hz, 2H), 7.45(AB quartet, 2H).

Precursor B - benzothiazole hydroxy nitrile

To a solution of 1 equivalent of 2-chloro-6-alkoxybenzothiazole (C.G. Stuckwisch J. Am. Chem. Soc., 1949, 3417.) in DMSO (1 M) was added 5 eq of sodium cyanide. The solution was heated to 80°C for 8 h and then allowed to cool to rt overnight. After workup, the residue was purified by column chromatography using hexanes:ethyl acetate to form the cyano benzothiazole in 60% yield. The compound where R was methyl was identical to an authentic sample purchased from Aldrich Chemical Company.

The cyano benzothiazole (1 eq) was dissolved in methylene chloride (1 M solution), cooled to 0°C and treated with 1.5 eq of boron tribromide or excess boron trichloride gas. After allowing the solution to warm to rt overnight, the solvent was removed in vacuo and the residue was purified by chromatography using hexanes:ethyl acetate to produce benzothiazole hydroxy nitrile in 75% yield. The

spectrum of this material was identical to material prepared by reaction with pyridinium hydrochloride (methyl ether case)

EXAMPLE 5

Synthesis Of Luciferin And
Its Analogs From Precursors

Precursors A and B synthesized above were each separately reacted with a substituted cysteine according to the method of White J. Am. Chem. Soc., 1969,91,2178, incorporated herein by reference, to form luciferin or a luciferin analog.

EXAMPLES 6A - 6C
Coupling of Activated Hypericin and
Porphyrin Analogs with Luciferin Analogs
to Form the Tethered Chemicals

6A - From the anhydride of hypericin diacid

To a 0.5 M solution of hypericin anhydride (1.0 eq) in 1:1 methylene chloride/DMF at 0 °C is added a solution of the luciferin analog (1.1 eq). The solution is allowed to stir to rt over 5 hours. The solvents are removed in

vacuo to afford the crude tethered molecule plus a small amount of luciferin analog. The crude product is partitioned between cold saturated sodium bicarbonate and ether. The aqueous layer is acidified with cold 1 M HCl to afford the tethered molecule as an amorphous solid.

6B - From bis-bromomethyl hypericin

To a 1 M solution of luciferin analog (1 eq) in 1:1 methylene chloride/DMF at 0 °C is added sodium hydride (1.0 eq). The suspension is stirred at 0 °C for 30 min and 1-2 M solution of bis-bromomethyl hypericin (1 eq) in DMF is added at a rate of approximately 1 mmol/second. The reaction is allowed to warm to rt over 5 hours. The tethered molecule is isolated by concentrating the solution in vacuo followed by trituration with ether.

6C - From activated porphyrins

To a solution of 1 eq of protoporphyrin IX commercially available from Aldrech Chemical Company in 1:1 methylene chloride/DMF at rt is added dicyclohexylcarbodiimide (1 eq), followed by the luciferin analog (1 eq). The solution is stirred at rt for 8 hours. The solvents are removed in vacuo and the crude product is treated with cold saturated sodium bicarbonate and filtered to remove dicyclohexylurea. The aqueous layer is carefully

acidified with cold 1 M HCl. The tethered molecule separates as an amorphous solid.

EXAMPLE 7

Expression of Luciferase under Control of EIAV Promoter

Luciferase was generated using a luciferase gene located in an expression plasmid constructed as explained below. The gene was under control of an EIAV long terminal repeat promoter. Plasmid DNA was introduced into equine cells in vitro and cell lysates were tested using functional assays of luciferase activity.

Construction of An Expression Plasmid Containing A Gene Encoding Luciferase And An EIAV Long Terminal Repeat Promoter

Materials and Methods

Plasmids. A complete proviral clone of the MA-1 isolate of EIAV in lambda EMBL4, designated EIAV 253, Carpenter et al., J. Vir. 65(3): 1605-1610 (1991), incorporated in its entirety herein by reference, obtained according to the methods disclosed in Carpenter et al. J. Vir., 65(3): 1605-1610 (1991), was digested with EcoR1 and the proviral insert was ligated to EcoR1 restricted pUC19

using conventional cloning techniques. A plasmid containing the complete EIAV provirus, p26A, was identified by colony blot hybridization and restriction endonuclease digestion. p26A was digested with BstN1 and Nar 1 and the 322 base pair (bp) fragment containing the complete MA-1 long terminal repeat (LTR) and 4 bp of flanking sequences was separated by electrophoresis and isolated by electroelution. See Carpenter et al., J Virol., 65(3): 1605-1610 (1991), Figure 1, incorporated herein by reference, for the partial sequence of MA-1, which is also incorporated by reference. The LTR is located at positions 7909-8231. The ends were filled in with the Klenow fragment of E. coli DNA polymerase I, modified by the addition of HindIII linkers, ligated to HindIII restricted pUC19, and the fragment was transformed into E. coli JM109. Colonies containing the LTR insert were identified as before and individual plasmids were purified by replating. Plasmid DNA was isolated by ion exchange chromatography and the LTR fragment was excised by HindIII digestion and purified by electrophoresis and electroelution. The LTR fragment was ligated to HindIII restricted pGL-Basic (Promega Biotec, Madison, WI), transformed into JM109, and positive colonies identified by blot hybridization. Plasmid DNA was isolated from twelve of the hybridization positive colonies and the orientation of the LTR insert with respect to the luciferase gene was determined by

restriction endonuclease digestion. Single clones containing the LTR insert in either the forward (pMA-1 LTR/LucF) or reverse (pMA-1LTR/LucR) orientation were selected for further analysis in functional assays of gene expression.

Additionally, a control plasmid having luciferase under control or the SV40 promoter, another plasmid encoding luciferase but no promoter, pGL2 Control and pGL2 Basic, were purchased from Promega Biotech (Madison, WI) for use in a luciferase assay. The plasmid, pRS Etat M, was provided by Dr. David Derse, National Cancer Institute, Frederick, MD. This plasmid expresses the EIAV transactivating protein, Tat, under the control of the Rous sarcoma virus promoter.

Transfection. Cells used for these studies included equine dermal (ED) cells (ATCC CCL57) and ED cells chronically infected with the MA-1 isolate of EIAV Carpenter et al., J. Virol., 65(3): 1605-1610 (1991) and Carpenter et al. J. Virol., 63: 2492-2496 (1989), both of which are incorporated in their entirety by reference. For analysis of luciferase expression, cells were seeded in 60 mm tissue culture plates at $5X10^5$ cells/plate and

transfected the following day using Transfectase reagent (BRL). In most cases, cells were transfected with 10ug of the luciferase expression plasmids (pMA-1 LTR/LucF, pMA-1 LTR/LucR, pGL2 control, or pGL2 basic) in the presence or absence of 100-300 ng pRS Etat-M. At 48 hr post-transfection, cells were lysed and assayed for luciferase expression using commercially available reagents (Promega). Comparable lysis buffers and assay reagents prepared in the laboratory were also tested.

Luciferase Assay. Assays for luciferase activity measure the production of light following the addition of the substrate, luciferin. Ten ul of cell lysate was mixed with 350 ul of reaction buffer containing 25 mM glycylglycine, pH 7.8, 5mM ATP, 15 mM $MgSO_4$, pH 7.8, 4mM EGTA, and 1 mM DTT. The samples were placed in an SLM 8000C spectrofluorometer, injected with 200 ul of 0.2 mM luciferin in 25 mM glycylglycine, 15mM $MgSO_4$, 4 mM EGTA, 2 mM DTT, and light output was measured at 560 nm for 60 sec.

Results

Plasmids containing the MA-1 LTR upstream of the luciferase gene were obtained by standard cloning techniques as described above. Plasmids were functionally characterized in ED cells, in MA-1 infected ED cells, and

in ED cells co-transfected with EIAV Tat (ETat). Only baseline levels of luciferase were expressed in ED cells in the absence of either virus infection or virus replication (Figure 2). Expression of luciferase was detectable in both virus-infected cells, and in cells co-transfected with ETat. The values obtained from these experiments were extrapolated to a standard curve derived using known molar concentrations of commercially available luciferase (Sigma, St. Louis, MO). The molar concentrations of luciferase in the cell lysates ranged between $1.8X10^{-13}$ to greater than $3X10^{-12}M$. The relatively low levels of luciferase are likely due to the low transfection efficiency of ED cells (Carpenter, unpublished observations).

While the invention has been described with reference to specific embodiments, it will be apparent to those skilled in the art that many alternatives, modifications, and variations may be made. Accordingly, it is intended to embrace all such alternatives, modifications, and variations that may fall within the spirit and scope of the appended claims.

What is claimed is:

1. A composition for inactivating a virus comprising:

a. a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range, selected from the group consisting of hematoporphyrin analogs, hypericin or other quinones, phthalocyanines and porphyrins;

b. an energy donating chemical that, when activated, tranfers energy or emits light within the range of that absorbed by said photosensitizing chemical; and

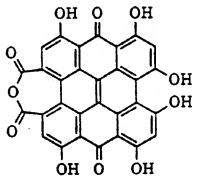
c. chemical means for activating the transfer of energy or the emission of light from said energy donating chemical.

2. The composition of claim 1 wherein a molecule of said photosensitizing chemical and a molecule of said energy donating chemical connected to each other by a chemical tether.

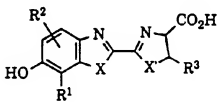
3. The composition of claim 2 wherein said photosensitizing chemical is a quinone or porphyrin and said chemical is luciferin or an analog thereof.

4. The composition of claim 3 wherein said photosensitizing chemical is hypericin or an analog thereof.

5. The composition of claim 4 wherein said hypericin analog has the following formula:



6. The composition of claim 5 wherein said luciferin analog has the following formula:



wherein R_1, R_2, R_3 or R_4 is said tether, wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4;

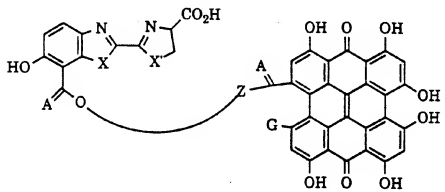
if R_1 is said tether, R_1 is $-CO_2(CH_2)_nY$, wherein n is 2 to 15; R_2-R_4 are H; and Y is OH, SH, NH_2;

if R_2 is said tether, R_2 is $-(CH_2)_nCO_2H$ and $-S(CH_2)_{n+1}Y$, wherein n is 1-15; R_1 and R_3 - R_4 are H; and Y is OH, NH_2 or SH;

if R_3 is said tether, R_3 is selected from the group consisting of $-CO_2H$ and $-(CH_2)_nY$, wherein n is 1-15; R_1, R_2 and R_4 are H; and Y is OH, NH_2 or SH; and

if R_4 is said tether, R_4 is $-(CH_2)_nY$, wherein n is 2-15; R_1-R_3 are H; and Y is OH, NH_2 or SH.

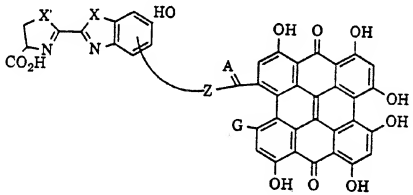
7. The composition of claim 6 wherein said photosensitizing chemical and said energy donating chemical comprise a tethered compound having the following formula:



wherein R_1 is said tether and X and X' are defined as in claim 6; and

wherein R_1 is $-CO_2(CH_2)_nZ$, wherein n is 2 to 15; R_2-R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me or CH_2Br.

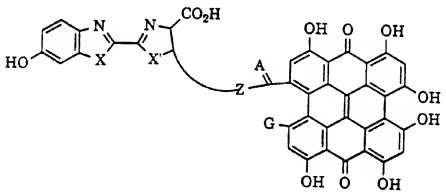
8. The composition of claim 6 wherein said photosensitizing chemical and said energy donating chemical comprise a tethered compound having the following formula:



wherein R_2 is said tether, and X and X' are defined as in claim 6; and

wherein R_2 is selected from the group consisting of $-(CH_2)_nCOZ$ and $-S(CH_2)Z$, wherein n is 1 to 15; R_1, R_3 and R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me or CH_2Br.

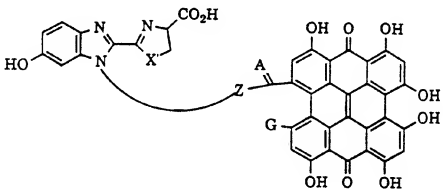
9. The composition of claim 6 wherein said photosensitizing chemical and said energy donating chemical comprise a tethered compound having the following formula:



wherein R_3 is said tether and X and X' are defined as in claim 6; and

R_3 is selected from the group consisting of -COZ and $-(CH_2)_nZ$, wherein n is 1 to 15; R_1-R_2 and R_4 are H; Z is O, NH, or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me or CH_2Br.

10. The composition of claim 6 wherein said photosensitizing chemical and said energy donating chemical comprise a tethered compound having the following formula:



wherein R_4 is said tether and X is defined as in claim 6; and

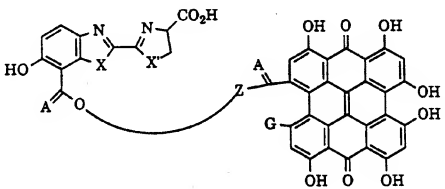
R_4 is $-(CH_2)_nZ$, wherein n is 2 to 15; R_1-R_3 and R_4 are H; Z is O NH, or S; A is O or H,H, and G is CH_3, CO_2H, CO_2Me or CH_2Br.

11. The composition of claims 7-10 wherein said chemical means for activating said luciferin or an analog thereof comprises an expression plasmid containing a gene encoding luciferase.

12. The composition of claim 11 wherein said gene is under the control of a promoter that is transactivated by replication of said virus.

13. The composition of claim 12 wherein said virus is a lentivirus and wherein said promoter is a lentivirus long terminal repeat or a portion thereof.

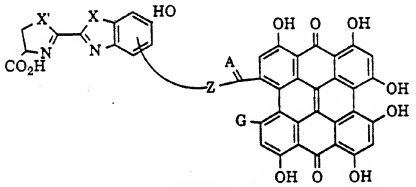
14. A tethered compound having the following formula:



wherein X and X' are selected from the group consisting of S, O, N, H,H, CH=CH or NR_4; and

wherein R_1 is said tether and is $-CO_2(CH_2)_nZ$, wherein n is 2 to 15; R_2-R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H or CO_2Me or CH_2Br.

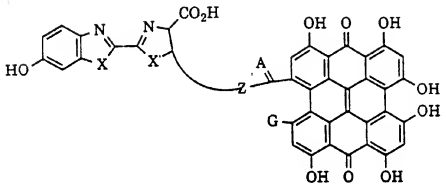
15. A tethered compound having the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_2 is said tether and is selected from the group consisting of $-(CH_2)_nCOZ$ and $-S(CH_2)_nZ$, wherein n is 1-15; R_1, R_3 and R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

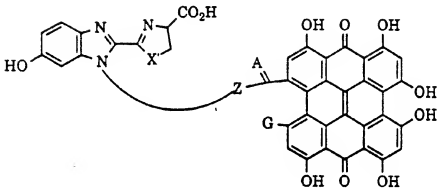
16. A tethered compound having the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_3 is said tether and is selected from the group consisting of -COZ and $-(CH_2)_nZ$, wherein n is 1-15; R_1, R_2 and R_4 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

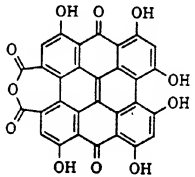
17. A tethered compound having the following formula:



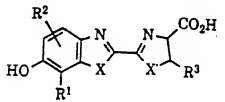
wherein X is selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_4 is said tether and is $-(CH_2)_nZ$, wherein n is 2-15; R_1-R_3 are H; Z is O, NH, S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

18. A hypericin analog for use as an intermediate in the preparing a tethered compound of claims 14-17 having the following formula:



19. A luciferin analog for use as an intermediate in preparing a tethered compound of claims 14-17 having the following formula:



wherein R_1, R_2, R_3, or R_4 represents said tether, wherein X and X' are selected from the group consisting of

S, O, H,H, CH=CH or NR_4;

if R_1 is said tether, R_1 is $-CO_2(CH_2)_nY$, wherein n is 2 to 15; R_2-R_4 are H; and Y is OH, NH or SH;

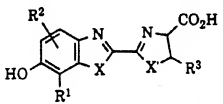
if R_2 is said tether, R_2 is selected from the group consisting of $-(CH_2)_nCO_2H$ and $-S(CH_2)_{n-1}Y$, wherein n is 1-15; R_1 and R_3 - R_4 are H and Y is OH, NH_2 or SH;;

if R_3 is said tether, R_3 is selected from the group consisting of $-CO_2H$ and $-(CH_2)_nY$, wherein n is 1-15; R_1-R_2 and R_4 are H; and Y is OH, NH_2 or SH; and

if R_4 is said tether, R_4 is $-(CH_2)_nY$, wherein n is 2-15; R_1-R_3 are H; and Y is OH, NH_2 or SH.

20. A method of synthesizing the tethered compound of claim 14 comprising the step of:

condensing hypericin or its analogs with a luciferin analog of the following formula:

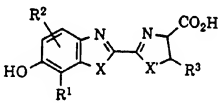


wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_1 is said tether and is $-(CH_2)_nCO_2H$ wherein n is 1-15; R_1 and R_3 - R_4 are H; and Y is OH, NH_2 or SH.

21. A method of synthesizing the tethered compound of claim 15 comprising the step of:

condensing hypericin or its analogs with a luciferin analog of the following formula:

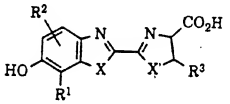


wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_2 is said tether and is selected from the group consisting of $-(CH_2)_nCO_2H$ and $-S(CH_2)_{n-1}Y$, wherein n is 1-15; R_1 and R_3-R_4 are H; and Y is OH, NH_2, or SH.

22. A method of synthesizing the tethered compound of claim 16 comprising the step of:

condensing hypericin or its analogs with a luciferin analog of the following formula:

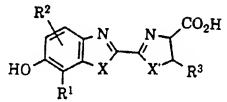


wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_3 is said tether and is selected from the group consisting of $-CO_2H$ and $-(CH_2)_nY$, wherein n is 1-15; R_1-R_2 and R_4 are H; Y is OH, NH_2, or SH.

23. A method of synthesizing the tethered compound of claim 17 comprising the step of:

condensing hypericin or its analogs with a luciferin analog of the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

R_4 is said tether and is $-(CH_2)_nY$, wherein n is 2-15; R_1-R_3 are H; and Y is OH, HN_2, or SH.

24. An expression plasmid for inactivating a virus comprising a luciferase gene under control of a promoter, wherein replication of said virus transactivates said promoter.

25. The expression plasmid of claim 24 wherein said virus is a lentivirus and said promoter is the lentivirus long terminal repeat or a portion thereof.

26. A liposome containing the expression plasmid of claim 24.

27. A cell transfected with the liposome of claim 26.

28. An eukaryotic cell containing a stably integrated copy of the expression plasmid of claim 24.

29. A viral vector produced from the eukaryotic cell of claim 28.

30. A composition for treating tumors by destroying tumor cells comprising:

a. a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range selected from the group consisting of quinones, porphyrins, and hypericin;

b. an energy donating chemical that, when activated, transfers energy or emits light within the range of that absorbed by said photosensitizing chemical; and

c. chemical means for activating the transfer of energy or emission of light from said energy donating chemical.

31. The composition of claim 30 wherein said photosensitizing chemical and said energy donating chemical are linked by a chemical tether.

32. The composition of claim 31 wherein said chemical is luciferin or a analog thereof and said photosensitizing chemical is a porphyrin.

33. The composition of claim 32 wherein said chemical activating means comprises an expression plasmid containing a gene encoding luciferase or another activating chemical.

34. The composition of claim 33 wherein said gene is under control of a promoter that is transactivated by elevated levels of proteins expressed by said tumor cells.

35. An expression plasmid for destroying tumor cells comprising a luciferase gene under control of a promoter, wherein said promoter is transactivated by elevated levels of proteins expressed by said tumor cells.

36. A liposome containing the expression plasmid of claim 35.

37. A cell transfected with the liposome of claim 36.

38. An eukaryotic cell containing a stably integrated copy of the expression plasmid of claim 35.

39. A viral vector produced from the eukaryotic cell of claim 38.

40. A method for inactivating a virus comprising the steps of:

a. introducing, into a cell infected by said virus, DNA encoding an activating chemical, wherein replication of said virus increases expression of said activating chemical.

b. introducing, into said cell, a compound comprising a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range selected from the group consisting of hematoporphyrin analogs, purpurins, phthalocyanines and porphyrins; and an energy donating chemical that, when activated by said activating chemical, emits light or energy of a wavelength within said known range, wherein said photosensitizing chemical is option-

ally connected to said energy donating chemical by a chemical tether.

41. The method of claim 40 wherein said DNA is located on an expression plasmid, and wherein said DNA is under control of a promoter, which is transactivated by replication of said virus.

42. The method of claim 41 wherein said expression plasmid is in a liposome.

43. The method of claim 40 wherein said DNA is located on a viral vector.

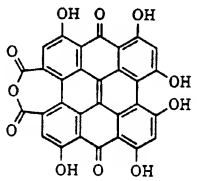
44. The method of claim 41 wherein a copy of said expression plasmid is stably integrated into an eukaryotic cell.

45. The method of claim 44 wherein said eukaryotic cell produces a viral vector containing said DNA.

46. The method of claim 40 wherein said DNA introduction and said compound introduction occurs in vivo.

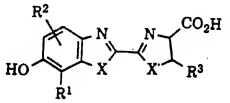
47. The method of claim 40 wherein said DNA introduction and said compound introduction occurs ex vivo.

48. The method of claim 40 wherein said photosensitizing chemical is a hypericin analog having the following formula:



49. The method of claim 48 wherein said energy donating chemical is luciferin or an analog thereof and said activating chemical is luciferase.

50. The method of claim 49 wherein said luciferin or its analogs have the following formula:



wherein R_1, R_2, R_3 and R_4 represents said tether; wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4;

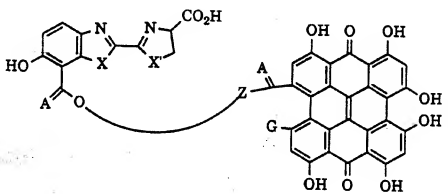
if R_1 is said tether, R_1 is $-CO_2(CH_2)_nY$, wherein n is 2 to 15; R_2-R_4 are H; and Y is OH, NH_2, or SH;

if R_2 is said tether, R_2 is selected from the group consisting of $-(CH_2)_nCO_2H$ and $-S(CH_2)_{n+1}Y$, wherein n is 1-15; R_1 and R_3 - R_4 are H; and Y is OH, NH_2, or SH; and

if R_3 is said tether, R_3 is selected from the group consisting of $-CO_2H$ and $-(CH_2)_nY$, wherein n is 1-15; R_1, R_2 and R_4 are H; and Y is OH, NH_2, or SH;

if R_4 is said tether, R_4 is $-(CH_2)_nY$, wherein n is 2-15; R_1-R_3 are H; and Y is OH, NH_2, or SH.

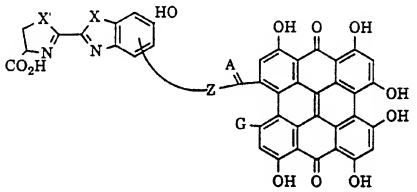
51. The method of claim 50 wherein said photosensitizing chemical and said luciferin or an analog thereof are connected by a chemical tether, wherein said tethered compound has the following formula:



wherein X is selected from the group consisting of S, O, H,H, CH=CH or NR_4;

wherein R_1 is said tether and is $-CO_2(CH_2)_nZ$, wherein n is 2 to 15; R_2-R_4 are H; Z is O, NH, or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

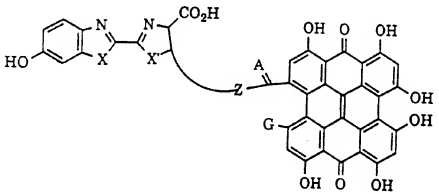
52. The method of claim 50 wherein said photosensitizing chemical and said luciferin or a analog thereof are connected by a chemical tether, wherein said tethered compound has the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_2 is said tether and is selected from the group consisting of $-(CH_2)_nCOZ$ and $-S(CH_2)_{n+1}Z$, wherein n is 1-15; R_1 and R_3-R_4 are H; Z is O, NH, or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

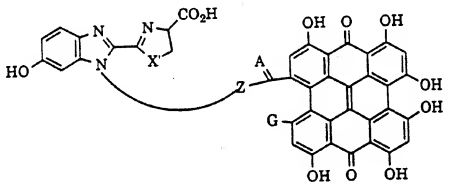
53. The method of claim 50 wherein said photosensitizing chemical and said luciferin or an analog thereof are connected by a chemical tether, wherein said tethered compound has the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

wherein R_3 is said tether and is selected from the group consisting of -COZ and $-(CH_2)_nZ$, wherein n is 1-15; R_1-R_2 and R_4 are H; Z is O, NH or S; A is O or H,H; G is CH_3, CO_2H, CO_2Me, or CH_2Br.

54. The method of claim 50 wherein said photosensitizing chemical and said luciferin or an analog thereof are connected by a chemical tether, wherein said tethered compound has the following formula:



wherein X and X' are selected from the group consisting of S, O, H,H, CH=CH or NR_4; and

R_4 is said tether and is $-(CH_2)_nZ$, wherein n is 2-15; and R_1-R_3 are H; Z is O, NH or S; A is O or H,H; and G is CH_3, CO_2H, CO_2Me, or CH_2Br.

55. A method for destroying a neoplastic cell comprising the steps of:

a. introducing, into said neoplastic cell, DNA encoding an activating chemical, wherein the elevated levels of a protein expressed in said tumor cells increases expression of said activating chemical;

b. introducing, into said neoplastic cell, a compound comprising a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range selected from the group consisting of porphyrins, hypericins and other quinones; and an energy donating chemical that, when activated by said activating chemical, emits light at a wavelength within said range, wherein said

photosensitizing chemical is optionally connected to said energy donating chemical by a chemical tether.

56. The method of claim 55 wherein said DNA is located on an expression plasmid.

57. The method of claim 56 wherein said expression plasmid is in a liposome.

58. The method of claim 56 wherein said expression plasmid is stably integrated into an eukaryotic cell.

59. The method of claim 58 wherein said eukaryotic cell produces a viral vector containing said DNA.

60. The method of claim 55 wherein said DNA introduction and said compound introduction occurs in vivo.

61. The method of claim 55 wherein said DNA introduction and said compound introduction occurs ex vivo.

62. The method of claim 55 wherein said energy donating chemical is luciferin and said activating chemical is luciferase.

63. The method of claim 62 wherein said photosensitizing chemical is a porphyrin.

64. A composition for inactivating a virus comprising:

a. a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range, selected from the group consisting of hematoporphyrin analogs, hypericin or other quinones, phthalocyanines and porphyrins; and

b. an energy donating chemical that, when activated, tranfers energy or emits light within the range of that absorbed by said photosensitizing chemical.

65. The composition of claim 64, wherein said photosensitizing chemical and said energy donating chemical are connected to each other by a chemical tether.

66. A composition for treating tumors by destroying tumor cells comprising:

a. a photosensitizing chemical activated by absorbing light or energy within a specific wavelength range selected from

the group consisting of quinones, porphyrins, and hypericin; and

b. an energy donating chemical that, when activated, transfers energy or emits light within the range of that absorbed by said photosensitizing chemical.

67. The composition of claim 66, wherein said photosensitizing chemical and said energy donating chemical are connected to each other by a chemical tether.

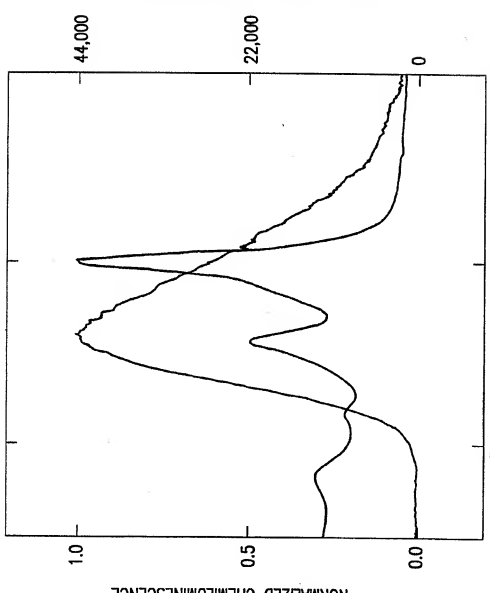

NORMALIZED CHEMILUMINESCENCE
1.0
0.5
0.0
500
600
700
WAVELENGH (nm)
44,000
22,000
0
MOLAR EXTINCTION COEFFICIENT OF HYPERICIN ($M^{-1}cm^{-1}$)

FIG.1

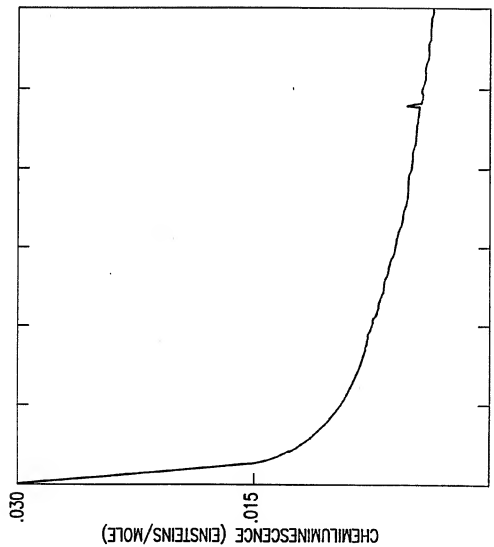

.030
.015
0
CHEMILUMINESCENCE (EINSTEINS/MOLE)
0
50
100
150
200
250
300
TIME (MIN)

FIG.2

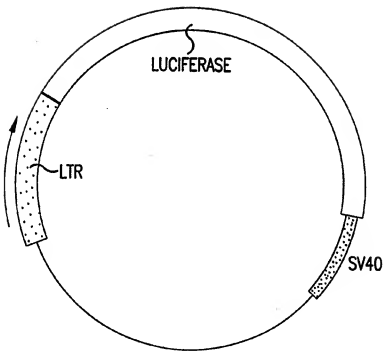


FIG.3

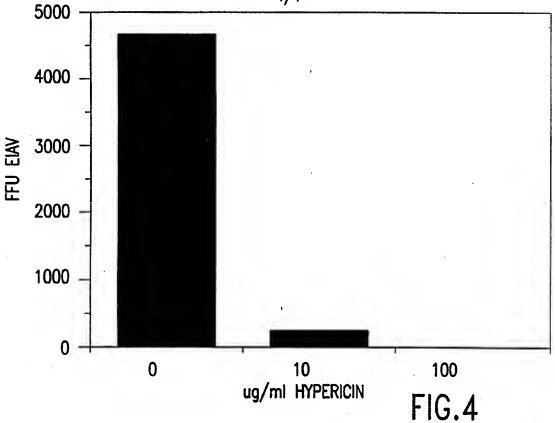

5000
4000
3000
2000
1000
0
FFU EIAV
0
10
100
ug/ml HYPERICIN

FIG.4

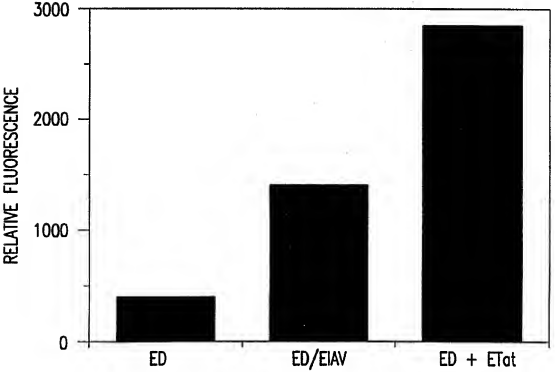

3000
2000
1000
0
RELATIVE FLUORESCENCE
ED
ED/EIAV
ED + ETat

FIG.5

INTERNATIONAL SEARCH REPORT

Inte. ational application No.
PCT/US93/12561

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/00, 15/09, 15/31, 15/63, 15/65, 15/87; C12Q 1/66
US CL :435/5, 6, 69.1, 172.1, 172.3, 320.1, 7.91, 8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 6, 69.1, 172.1, 172.3, 320.1, 7.91, 8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Medicine, Medline
Search Terms: hypericin, luciferin, inactivation, virus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences, Vol. 85, issued July 1988, D. Meruelo et al., "Therapeutic Agents with Dramatic Antiretroviral Activity and Little Toxicity at Effective Doses: Aromatic Polycyclic Diones Hypericin and Pseudohypericin", pages 5230-5234, see Figure 4.	1-67
A	Photochemistry and Photobiology, Vol. 43, No. 2, issued November 1986, Van Steveninck et al., "Photodynamic Effects Induced by the Luciferin/Luciferase System", pages 213-216, see Abstract.	1-67

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
03 MARCH 1994	11 MAR 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DAVID GUZO Jill Warden for
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/12561

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences, Vol. 86, issued August 1989, G. Lavie et al., "Studies of the Mechainism of Action of the Antiretroviral Agents Hypericin and Pseudohypericin", pages 5963-5967, see page 5966.	1-67
A	Biochemical and Biophysical Research Communications, Vol. 172, No. 1, issued 15 October 1990, G.A. Kraus et al., "Antiretroviral Activity of Synthetic Hypericin and Related Analogs", pages 149-153, see page 151.	1-67